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COPULATORY PATTERN AND FERTILIZATION SUCCESS IN MALE WOLF SPIDERS WITHOUT PRE- OR POST-COPULATORY SPERM INDUCTION

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ABSTRACT. Experiments with *Lycosa malitiosa* Tullgren 1905 were carried out to determine: a) whether males that had never performed sperm induction can copulate, b) whether these males perform an altered copulatory pattern, and c) whether the stored sperm from a single sperm induction is enough to inseminate two consecutive females. A group of males whose genital pores were sealed with melted paraffin immediately after molting copulated once; then, the seal was removed, and later these males copulated again. A second group of males was untreated prior to their first copulation but then immediately had their genital pores sealed and subsequently were allowed to copulate again. Two other groups of males were used as controls: their genital pores were "pseudosealed" by having paraffin placed beside them. All females were virgins, and the number of progeny produced by each was recorded. Males that never had sperm in their palps maintained the basic species-specific copulatory pattern, although they showed several minor copulatory alterations. The second copulation of males prevented from recharging their palps resulted in the production of abundant progeny. Matings of older males (second copulations) resulted in a similar number of spiderlings as that of younger males (first copulations).

Since Petrunkevitch (1911) some authors have assumed that the presence of sperm filling the palpal duct would be indispensable for male spiders to initiate sexual activities (see review in Rovner 1966). More than 50 years later Rovner (1966) experimentally demonstrated that this assumption was not true for the lycosid *Rabidosa rabida* (Walckenaer 1837). This author observed courtship in males where sperm induction was prevented by several methods: induced palpal autotomy, sealing genital pore, sealing spinnerets, or fixing the palps on the cephalothorax. Those earlier assumptions were probably based on observations of recently molted males, during the short period in which males do not court. Male spiders usually perform an initial sperm induction before copulation, although some linyphiids do it only after completing a series of insertions early in copulation (Rovner 1967).

The aim of this study was to test if copulation takes place using males with "empty" palps and, if it occurs, whether the copulatory pattern is altered. Also, the study examines if the amount of sperm originally stored in the palps is sufficient to assure the success of a second copulation (i.e., when sperm induction is prevented following the first copulation).

Experiments were carried out using individuals of *Lycosa malitiosa* Tullgren 1905, a common large-sized wolf spider from southern Uruguay. Its sexual behavior, brood size and reproductive strategy, as well as its phenology, are well known (Costa 1975, 1979, 1991; Costa & Capocasale 1984, 1985; Capocasale et al. 1984).

METHODS

Subadults of *Lycosa malitiosa* were collected in Marindia, Canelones, Uruguay, during Fall 1987 and raised to adults in the laboratory. Forty-eight adult males and 83 adult females were used. Spiders were kept in individual cages and mainly fed with *Tenebrio* sp. larvae (Coleoptera). For other rearing details see Costa (1979) and Costa & Capocasale (1984). For male-female encounters, males were introduced in a wide arena (cylindrical cage of 18 cm diameter), where the female had been placed one or more days before.

Adult males were assigned to four groups (I, II, III and IV). Males observed during molting process were randomly placed in groups I or III; the other males were randomly placed in groups II or IV. Each group was initially composed of 12 males. Every male was involved in two experimental phases: Phase

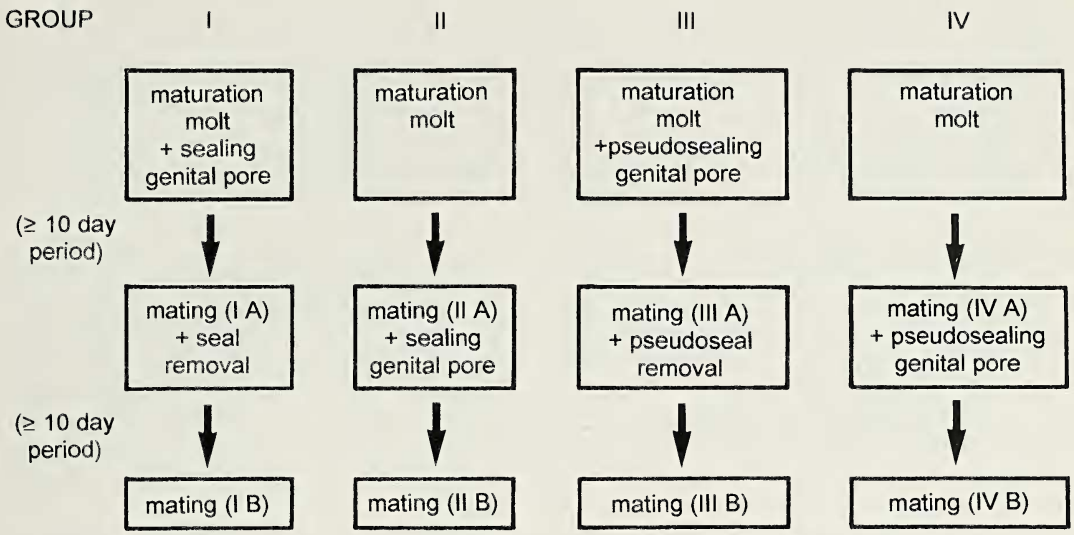

Figure 1.—Diagrammatic representation of the experimental design involving male *Lycosa malitiosa*. Males of each group copulated first (subgroup A) ten or more days after the final molt, and copulated again (subgroup B) ten or more days after their first copulation.

A, when males mated for the first time, and Phase B, when males copulated again. Thus, a total of eight copulation subgroups were established: IA, IB, IIA, IIB, IIIA, IIIB, IVA and IVB (Fig. 1). All females used were virgins.

Group I.—Subadult males were monitored as long as possible to observe their maturation molt. When ecdysis was completed, the spider was observed for one hour to permit the hardening of the new cuticle and to ensure that sperm induction did not occur. Males were anaesthetized with CO_2 and their genital pores were sealed using melted paraffin. Ten or more days after molting, males engaged in their first copulation (subgroup IA). Immediately after copulation, these males were anaesthetized with CO_2 and the seal was removed. Ten or more days after their first mating these males mated again with other virgin females (subgroup IB).

Group II.—In subgroup IIA, males copulated 10 or more days after their maturation molt; immediately after copulation, males were anaesthetized and their genital pores were sealed. Ten or more days later, they had a second copulation with a virgin female (subgroup IIB).

Group III.—Subadult males were watched until their maturation molt. Newly-emerged males were observed for one hour to ensure against sperm induction. Then they were anaesthetized and melted paraffin was placed beside the genital pore ("pseudoseal"), avoiding sealing it. Ten or more days after pseudosealing, males of this IIIA subgroup copulated with virgin females. They were immediately anaesthetized and the pseudoseal was removed. Ten or more days after the first mating, these males (subgroup IIIB) remated with virgin females.

Group IV.—Males copulated 10 or more days after their maturation molt (subgroup IVA) and immediately were anaesthetized and pseudosealed. Ten or more days after their first copulation, these males (subgroup IVB) recopulated with virgin females.

A schematic representation of the palpal condition of the eight experimental subgroups is given in Table 1. One male from group I died of natural causes before his first copulation. Second copulations were less numerous than were first copulations: they diminished by three in group I, three in group II, four in group III and one in group IV. This diminution was caused by unsuccessful courtship (three cases), male deaths due to natural causes (four cases), female bite (three cases), and accidental damage during manipulation (one case).

The course of copulatory behavior was recorded on forms that organized data on general copulatory pattern, alternation in the use of the palps, and number and duration of pal-

Table 1.—Male palpal condition before mating in the experimental groups. For experimental design see Figure 1.

Male group	I	II	III	IV
Phase A	without sperm	with sperm	control (with sperm)	with sperm
Phase B	with sperm	sperm not replaced	with sperm	contral (with sperm)

pal insertions. As described by Costa (1979) and Costa & Sotelo (1994), two main copulatory phases occur in *L. malitiosa*: Pattern I (PI) consists of multiple consecutive insertions with the same palp, change of side, multiple insertions with the other palp, and so on. Pattern II (PII) follows PI and consists of alternate use of the palps after a single insertion. "Brief" insertions consisted of the palp engaging in the epigynum, complete hematodochal distension with simultaneous spine erection, then immediate disengagement, collapse of the hematodocha, and rapid spine descent. I considered as "many" brief insertions the occurrence of more than 20 in a copulation; and "few", between 5–20 in a copulation (less than five was not considered). Pseudoinsertions, if the palp disengaged from the female epigynum before complete swelling of the hematodocha and/or complete spine erection, were not counted as insertions.

Males were sacrificed immediately after their second copulation, using carbon tetrachloride vapors. Mated females were maintained in the laboratory. The numbers of both egg sacs and spiderlings were recorded. Juveniles were removed from the female's back after 10 days following their emergence, a time when they disperse in nature. One female from subgroup IVA died before completing reproduction and was not considered. Male and female voucher specimens were deposited in the arachnological collection of the Museo Nacional de Historia Natural, Montevideo.

In the analysis, groups I and II were compared with their respective controls, III and IV, always within the same experimental phase (A or B). In some cases both phases were compared between them within the same group. Both two-tailed statistics Student *t*-test and Mann-Whitney *U*-test were used.

RESULTS

Copulatory characteristics of the experimental groups are given in Table 2. Copulation durations were similar among the groups. Only durations from subgroup IVA showed low values, particularly in comparison with subgroups IIA and IVB, but did not show significant differences using the Student *t*-test. Differences in copulation duration among the subgroups were not correlated with environmental temperature variations. The two shortest copulations correspond to low temperatures.

The species-specific pattern of copulation was basically maintained in all experimental groups. The number of total insertions did not show significant differences among groups using the *U*-test. However, the values from subgroup IA and especially subgroup IB were the highest. No differences among subgroups were found when comparing separately Pattern I or Pattern II. However, insertions were very numerous in both the PI and PII copulatory patterns of subgroup IB.

Modifications were numerous in subgroups IA and IB, while they were minimal in IIA. The more frequent modifications of the copulatory pattern were: (1) occurrence of "few" and "many" brief insertions (see Methods); (2) occurrence of pseudoinsertions; (3) Pattern II very short or absent; and (4) occurrence of some multiple consecutive insertions of the same palp intercalating Pattern II (Table 2). Modifications were numerous in subgroups IA and IB, while they were minimal in IIA.

The number of progeny produced by females is shown in Fig. 2. Considering the mean total number of spiderlings, high values were observed in subgroups IIA, IIIA, and IVB. Total juvenile number from IIA did not show a significant difference when compared with IVA ($U = 34$), although P was near the 0.05 level. No offspring were produced by females of subgroup IA. A low number of spiderlings was found in IB, reflecting the absence of progeny in four of the nine females. These values were just significant in relation to the IIIB values ($U = 15$; $P = 0.05$). Low progeny values of females of subgroup IVA

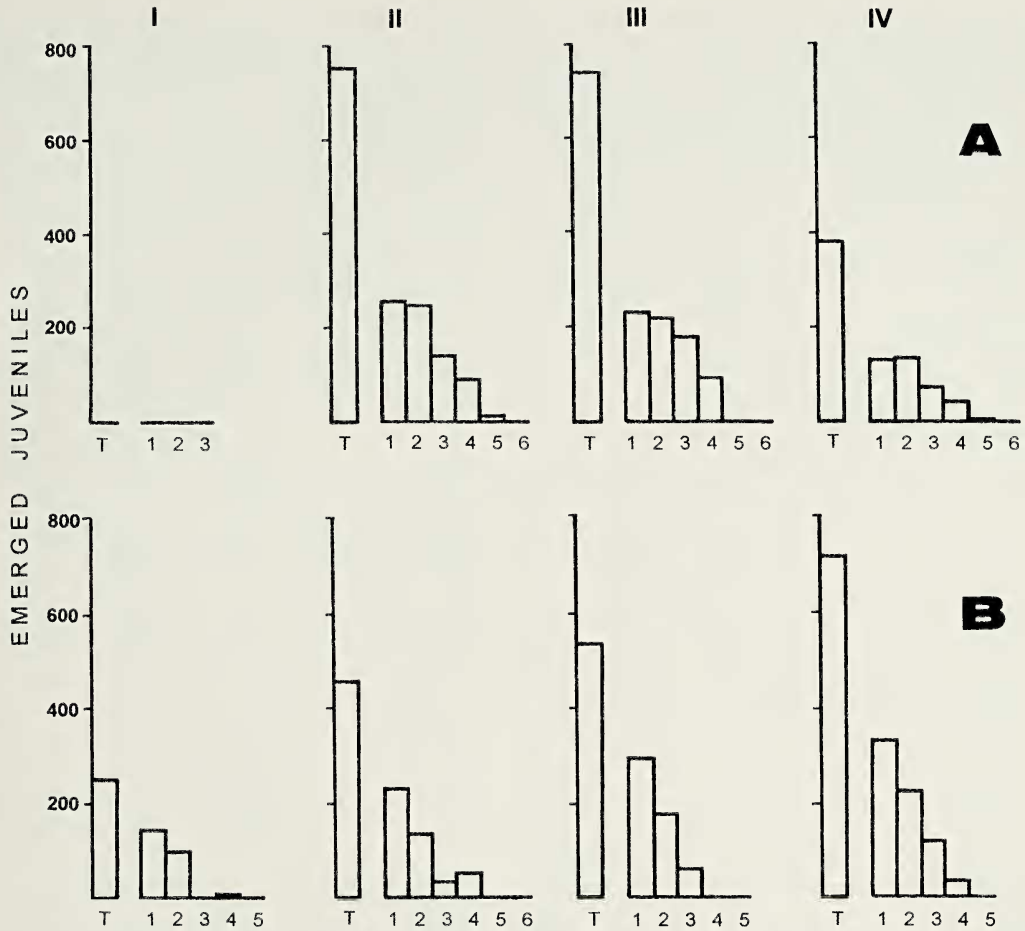

Figure 2.—Progeny from the four experimental female groups. Bars indicate mean values of spiderlings: total values (T) and number of spiderlings emerged from each egg sac (first = 1, second = 2, etc.). Zero values were included. Clutch numbers without bars indicate occurrence of egg sacs but no juveniles. For details of experimental design, see Figure 1.

also resulted in significant differences compared to subgroup IVB ($U = 25.5$; $P < 0.05$).

As to the copulatory pattern, the number of alterations in palpal insertions for each experimental subgroup (Table 2) showed an inverse correlation with the number of progeny produced ($r = -0.809$, $P < 0.05$). No differences among groups were found in either male or female age during copulation (Table 3). No differences in the number of progeny were found when comparing Phase A with Phase B within the same group, with the exception of IA *vs.* IB subgroups. (As a result of the experimental plan, males mating in Phase B were older than males mating in Phase A.)

The number of egg sacs varied between 4–6, except subgroup IA in which there was a mean of three egg sacs. The subgroup IA egg sacs were immediately eaten or abandoned.

All males, excepting the sealed IIB males, had a whitish drop in the genital pore region when examined after they were sacrificed.

DISCUSSION

Results show that male *Lycosa malitiosa* maintain full sexual activity despite the absence of sperm in their palps. These results agree with the observations of Rovner (1966, 1967) in lycosid and linyphiid spiders. Although the male copulatory behavior of subgroup IA followed the normal species-specific pattern (Costa 1979; Costa & Sotelo 1994), atypical copulations were frequent. Only four matings of 12 were completely typical, which

Table 2.—Summary of copulatory characteristics from experimental groups of *Lycosa malitiosa*. Each male performed two consecutive copulations (A and B), each with two virgin females. Abbreviations: Number of observed matings (# obs.); copulation duration (CD); copulatory patterns I and II (PI and PII); main copulatory alterations number: Brief insertions (BI: several and few), pseudoinsertions (pseudoins.), reduced pattern II (PII brief), and multiple insertions during pattern II (PII-MI).

Subgroup	IA	IB	IIA	IIB	IIIA	IIIB	IVA	IVB
# obs.	12	9	12	9	12	8	11	10
CD (min)								
Mean	68.7	76.7	75.6	72.7	76.7	79.1	59.1	77.8
SD	25.8	30.8	17.7	15.9	21.5	27.5	25.3	16.8
Temp. (°C)								
Mean	24.5	25.8	25.0	26.5	25.0	26.3	25.0	25.7
SD	2.3	2.8	3.0	2.1	2.3	2.3	2.7	2.4
Palpal insertions (Total)								
Mean	314.4	376.3	273.3	291.6	268.0	254.4	238.6	267.7
SD	82.3	174.8	53.8	77.9	75.7	120.8	95.8	94.5
PI								
Mean	271.8	309.8	225.6	244.6	215.5	207.6	196.5	221.7
SD	62.9	147.9	51.2	75.8	67.4	107.7	80.4	83.1
PII								
Mean	42.7	66.6	47.8	47.0	52.5	46.9	42.1	46.0
SD	24.5	43.1	15.8	22.2	13.7	16.0	27.8	21.9
Alterations in palpal insertions								
BI/Several	4	3	0	3	2	1	1	2
BI/Few	5	3	1	1	1	2	3	3
Pseudoins.	2	2	0	0	2	0	2	2
PII brief	3	1	0	1	0	0	3	1
PII-MI	5	4	1	4	3	2	1	3
Progeny								
Mean	0	249.0	753.8	453.0	737.8	529.5	381.7	716.1
SD	—	294.1	498.6	244.5	366.0	296.8	283.7	374.7
Females without progeny	12	4	0	1	0	0	2	0

Table 3.—Adult age (days post-final molt) of copulating males (M) and females (F) in the experimental groups of *Lycosa malitiosa*. *n* = number of copulations per group.

	Group							
	I		II		III		IV	
	M	F	M	F	M	F	M	F
Phase A								
Mean	39.1	13.9	40.4	21.3	41.4	15.0	40.8	28.1
SD	17.9	7.7	19.2	23.4	15.1	10.0	17.4	24.0
n	12		12		12		11	
Phase B								
Mean	79.4	10.7	91.3	15.4	85.3	10.4	83.5	22.2
SD	35.5	6.4	37.5	9.6	24.1	8.2	36.9	22.0
n	9		9		8		10	

might be attributed to the particular experimental procedure. However, other factors probably are involved, considering that males with sperm in their palps also showed atypical behaviors. The lack of progeny from IA females confirmed that sealing the male gonopore prevented sperm uptake by the palps completely. It also indicated that parthenogenesis does not occur in the studied population of *L. malitiosa*, as was suggested in the dysderid *Dysdera hungarica* Kulczynski 1897 (Deeleman-Reinhold 1986). Females of subgroup IA made unsuccessful egg sacs, as described also by Capocasale et al. (1984) for virgin females of this species.

Maintenance of the typical species-specific copulatory pattern in subgroup IA indicated that copulation is mainly performed independently of proprioceptive information generated by the presence of sperm in the palpal duct. Seminal fluid released into the palpal duct could substitute for the sperm and help to maintain the typical copulatory mechanics; however, copulatory maneuvers probably are determined primarily by neural centers. Rovner (1967) observed "pseudocopulations" similar to normal copulations in palpectomized males of *Linyphia triangularis* (Clerck 1757) (Linyphiidae).

Copulation duration did not show significant variations among experimental subgroups, including subgroup IA. Copulation duration in subgroup IVA was brief. This group also showed many alterations in the copulatory pattern and small number of progeny from females. Considering that the same males showed normal copulation and progeny in IVB, the result in IVA was surprising and could be attributed to chance.

The well-established relationship between copulation duration and environmental temperature in this species (Costa 1979; Costa & Sotelo 1984, 1994) did not determine the differences observed here in copulatory duration. Shortest copulations of subgroups IA and IVA disagree with the inverse relationship noted by these authors.

The fact that the greatest number of palpal insertions was performed by subgroup IB suggested some unknown influence of the application and removal of the genital pore seal. This subgroup showed a typical copulation duration, which may be explained by the short duration of many of these insertions (several "brief" insertions). The small number of progeny produced by IB females could be attributed to the occurrence of brief insertions and other copulatory alterations (see Table 2). However, subgroup IVB, which presented an occurrence of copulatory alterations similar to IB, generated abundant progeny. It is most likely that the seal removal procedure used in group I was imperfect, interfering with sperm induction in some males. The probability of incomplete seal removal was supported by the absence of offspring in four IB females, and a relatively small number of progeny (448.2 ± 247.8 spiderlings) in the other five females.

Subgroup IIB produced a moderate number of progeny. Males from this group had been prevented from recharging their palps after their first copulation (postcopulatory sealing of the genital pore). Their first copulation was normal and generated abundant progeny. Despite this "emptying", the "residual" sperm were sufficient in number to yield a relatively high number of spiderlings in IIB, especially considering that it involved a number of copulatory modifications. The sperm storage capacity of *L. malitiosa* males is large; and sperm remains viable during the long period of consecutive ovipositions, between 6–7 months in warm conditions (Costa & Capocasale 1985; Costa 1991). The abundance of sperm is confirmed by one particular observation: one male from subgroup IVA failed to insert its left palp during an entire mating that yielded 530 spiderlings. In *L. malitiosa*, the occurrence of multiple copulations in the females (Costa 1979) would primarily have advantages other than renewing the sperm supply (see review from Austad 1984).

The sperm droplet that was exuded onto the surface surrounding the male's genital pore after mating was not observed in other males which had not recently copulated and which had been similarly sacrificed. Perhaps sperm accumulates at the end of the male's genital duct stimulated by copulation, "waiting for" an immediate postcopulatory sperm induction.

Abundant progeny resulted from subgroups IIA, IIIA and IVB in numbers similar to those obtained by Capocasale et al. (1984) for female *L. malitiosa* reared under similar conditions. The other subgroups showed reductions, suggesting some influence of the experimental procedure. However other factors, such as cryptic female choice during copulation

(Eberhard 1994), could be acting and affecting each experimental subgroup differently.

Experimental groups III and IV were the controls for groups I and II; they provided tests for the effects of experimental manipulations (anaesthesia and paraffin application) on spider performance. Results from IIIA and IVB indicate that experimental manipulations did not affect either the copulatory characteristics or the number of progeny produced by the spiders. The unexpected copulatory modifications and low production of progeny occurring in IVA, cannot be explained. These males had filled their palps before copulation and followed an experimental procedure similar to subgroup IIA; also, they were the same individuals subsequently used for subgroup IVB, which had a normal number of progeny.

Female age was similar among subgroups, but the used method involved two different age classes of males, according their use in first or second matings. The average age of males during second copulations ranged between 80–90 days post-final molt, which was close to the age they normally died under laboratory conditions (101.3 ± 21.1 days post-final molt; Costa 1985). Male senility during attempted second copulations probably caused some of the female rejections observed during courtship. However, no significant differences were found in copulatory duration or pattern, nor between the number of progeny produced by first or second matings (group I was obviously excluded). The results for copulation duration in *L. malitiosa* do not agree with the positive correlation between both female and male age and copulation duration reported for the lycosid *Schizocosa ocreata* (Hentz 1844) (see Hebets & Uetz 1995).

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